

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 11 OCT 2005

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Applicant's or agent's file reference M80713536: JWC: KJM: ah	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/AU2004/000802	International filing date (day/month/year) 18 June 2004	Priority date (day/month/year) 20 June 2003	
International Patent Classification (IPC) or national classification and IPC			
Int. Cl. ⁷ C12N 15/29, 15/64			
Applicant MOLECULAR PLANT BREEDING NOMINEES LTD et al			

- This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 5 sheets, including this cover sheet.
- This report is also accompanied by ANNEXES, comprising:
 - ☐ (sent to the applicant and to the International Bureau) a total of sheets, as follows:
 - ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
 - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
 - ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
- This report contains indications relating to the following items:

<input checked="" type="checkbox"/> Box No. I	Basis of the report
<input type="checkbox"/> Box No. II	Priority
<input type="checkbox"/> Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input type="checkbox"/> Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/> Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/> Box No. VI	Certain documents cited
<input type="checkbox"/> Box No. VII	Certain defects in the international application
<input checked="" type="checkbox"/> Box No. VIII	Certain observations on the international application

Date of submission of the demand 19 April 2005	Date of completion of the report 4 October 2005
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Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This report is based on translations from the original language into the following language which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3 and 23.1 (b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
 - ☐ the international application as originally filed/furnished
 - ☒ the description:
 - pages 1-28 as originally filed/furnished
 - pages* received by this Authority on with the letter of
 - pages* received by this Authority on with the letter of
 - ☒ the claims:
 - pages as originally filed/furnished
 - pages* as amended (together with any statement) under Article 19
 - pages* 28-30 received by this Authority on 19/4/05 with the letter of 18/4/05
 - pages* received by this Authority on with the letter of
 - ☒ the drawings:
 - pages 1/9-9/9 as originally filed/furnished
 - pages* received by this Authority on with the letter of
 - pages* received by this Authority on with the letter of
 - ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
 - ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (specify):
 - ☐ any table(s) related to the sequence listing (specify):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
 - ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (specify):
 - ☐ any table(s) related to the sequence listing (specify):

* If item 4 applies, some or all of those sheets may be marked "superseded."

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-25	YES
	Claims	NO
Inventive step (IS)	Claims	YES
	Claims 1-25	NO
Industrial applicability (IA)	Claims 1-25	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The following citations from the search report are referred to in this report: Error! Bookmark not defined.

D1 = WO 1993/004174 A1 (The University of Melbourne). 4 March 1993. Error! Bookmark not defined.

The invention is directed to an isolated nucleic acid molecule comprising SEQ ID No's: 2 and 3 capable of modifying pollen-specific expression. The specification discloses the isolation of a pollen-specific promoter derived from the ryegrass gene Lol p1 and Lol p2 of *L. perenne* (Lolium) capable of modifying tissue-specific expression (pollen-specific), preferably of an operably-linked second nucleic acid molecule.

Inventive Step

D1 is the closest prior art document, which is directed to the use of a ryegrass pollen specific promoter from *L. perenne* (Lol p1), which is capable of modifying pollen specific expression. It discloses a recombinant DNA molecule comprising a promoter, directing synthesis of the Lol 1 allergen from the pollen of ryegrass *L. perenne*, which is a developmentally pollen specific expression vector. Furthermore it discloses a nucleotide sequence encoding a polypeptide having a deleterious function, which is transcribed by said pollen specific promoter. In the attorneys response it is suggested that D1 does not disclose the sequence of Lol p1, the examiner agrees. However D1 discloses the use of the Lol p1 promoter for pollen specific expression. As such claim 5 lacks an inventive step.

Furthermore D1 discloses that the present invention also extends to the promoters of ryegrass pollen proteins and that the skilled artisan will immediately recognise the importance of such promoters in selectively expressing a particular trait during pollen formation (see page 26 lines 18-31). In addition D1 discloses that allergens of Lol p1, p2, p3 and 5 have been extensively studied and that full amino acid sequences of lol p1 & 2 have been reported. Therefore a PSA in faced with the problem of finding pollen specific promoters for modifying pollen specific expression, would in reading D1, readily recognise the importance of Lol p1, 2, 3 and 5 promoters for said pollen specific expression. As such claims 1-25 lack inventive step. Although it is acknowledged that D1 does not specifically disclose the nucleic acid molecule of claim 22, a ribonuclease barnase, it is not considered inventive to simply use a molecule of interest as the operably-linked second nucleic acid molecule transcribed by the pollen-specific promoter for the down-regulation allergens. As such claim 22 lacks an inventive step.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 3 lacks descriptive support in relation to the disclosure of *Festuca* species.

Claim 3 is directed to an isolated nucleic acid molecule and a low allergy plant from *Festuca* species respectively. However there appears to be no descriptive support for *Festuca* species in the specification. **Error! Bookmark not defined.** Although it is acknowledged that *Festuca* species is mentioned in the specification, no sequences have been isolated, characterised or exemplified. As such without providing examples or evidence relating to *Festuca* species, for example isolated pollen-specific promoter sequences, claims 3 and 16 lack descriptive support.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

a. type of material

- ☒ a sequence listing
☐ table(s) related to the sequence listing

b. format of material

- ☒ in written format
☐ in computer readable form

c. time of filing/furnishing

- ☒ contained in the international application as filed
☐ filed together with the international application in computer readable form
☐ furnished subsequently to this Authority for the purposes of search and/or examination
☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."

CLAIMS

1. An isolated nucleic acid molecule including a sequence of nucleotides selected from the group consisting of (a) a nucleotide sequence set forth in SEQ ID NO:2 or 3; (b) a sequence which hybridises to SEQ ID NO:2 or 3 under moderately stringent or high stringency conditions; (c) a complement of (a) or (b); and (d) a fragment or variant of (a), (b) or (c);

wherein said molecule is capable of modifying pollen-specific expression.

2. An isolated nucleic acid molecule according to claim 1 wherein said molecule is capable of modifying pollen-specific expression of an operably-linked second nucleic acid molecule.

3. An isolated nucleic acid molecule according to claim 2 from a ryegrass (*Lolium*) or Fescue (*Festuca*) species.

4. An isolated nucleic acid molecule according to claim 3 from perennial ryegrass (*L.perenne*).

5. An isolated nucleic acid molecule according to claim 4 from a perennial ryegrass (*L. perenne*) *Lol p 1* gene.

6. An isolated nucleic acid molecule according to claim 4 from a perennial ryegrass (*L.perenne*) *Lol p 2* gene.

7. An isolated nucleic acid molecule according to claim 2 wherein said second nucleic acid molecule is capable of down-regulating expression of a pollen allergen.

8. An isolated nucleic acid molecule according to claim 7 wherein said pollen allergen is *Lol p 1* and/or *Lol p 2*.

9. A vector including a nucleic acid molecule according to claim 1.

10. A vector according to claim 9, further including a second nucleic acid molecule and a terminator, said nucleic acid molecule, second nucleic acid molecule and terminator being operably linked so as to result in expression of said second nucleic acid molecule.
- 5 11. A vector according to claim 10 wherein said second nucleic acid molecule is capable of modifying expression of a pollen allergen.
12. A vector according to claim 11 wherein said pollen allergen is *Lol p 1* and/or *Lol p 2*.
13. A chimeric gene including a nucleic acid molecule according to claim 10 1 operably linked to a second nucleic acid molecule.
14. A chimeric gene according to claim 13 wherein said second nucleic acid molecule is capable of modifying expression of a pollen allergen.
15. A chimeric gene according to claim 14 wherein said pollen allergen is *Lol p 1* and/or *Lol p 2*.
- 15 16. A plant cell, plant, plant seed or other plant part including a nucleic acid molecule according to claim 1, a vector according to claim 9 or a chimeric gene according to claim 13.
17. A low allergy plant including a nucleic acid molecule according to claim 1, a vector according to claim 9 or a chimeric gene according to claim 13.
- 20 18. A low allergy plant according to claim 17 which is a ryegrass or fescue.
19. A method of modifying gene expression in pollen said method including introducing into a plant cell an effective amount of a nucleic acid molecule according to claim 1, a vector according to claim 9 or a chimeric gene 25 according to claim 13.

20. A method of producing a plant with reduced male fertility compared with a wild-type plant, said method including introducing into the plant a nucleic acid molecule according to claim 1 in combination with a further nucleic acid molecule capable of modulating male fertility.

- 5 21. A method according to claim 20 wherein said further nucleic acid molecule is capable of modifying pollen development.

22. A method according to claim 21 wherein said further nucleic acid molecule encodes bacterial ribonuclease barnase.

23. A plant produced by a method according to claim 20.

- 10 24. A plant according to claim 23 wherein said plant is a male sterile plant.

25. A preparation for transforming a plant including a nucleic acid molecule according to claim 1.